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Competitor's Claim

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TITLE: Method of enriching rare cells

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CLAIMS:

What is claimed is:

1. A method for enriching cancer cells in a bodily fluid sample comprising cancer cells and non-rare cells comprising:

(a) obtaining the sample comprising cancer cells and non-rare cells;

(b) subjecting the sample to multiple density gradient separation comprising a first density gradient and a second density gradient, wherein the second density gradient is greater than the first density gradient, and producing a first fluid comprising an increased concentration of cancer cells of a first density, and a second fluid

comprising an increased concentration of cancer cells of a second density, wherein
the second density is greater than the first density;

wherein subjecting the sample to multiple density gradient separation includes
producing a plasma layer, a first interface layer, a first gradient layer, a second
interface layer, a second gradient layer, and a cell pellet;

wherein producing the first fluid includes combining the first interface layer and
the first gradient layer and forming a first suspension; and

wherein producing the second fluid includes combining the second interface layer and
the second gradient layer and forming a second suspension;

(c) subjecting said second fluid comprising the second suspension to a binding agent
that binds non-rare cells;

(d) removing the bound non-rare cells from the second fluid to provide a second
fluid enriched with the greater density cancer cells, and

(e) preparing a fluid enriched with the cancer cells of the first density and the
cancer cells of the greater density by combining the cancer cells of the first
density from the first fluid and the greater density cancer cells from the second
fluid enriched with the greater density cancer cells.

2. The method of claim 1, wherein said cancer cells are alive during the course of
said method.

3. The method of claim 1, wherein said cancer cells are epithelial cells.

4. The method of claim 3, wherein said epithelial cells are prostate cancer cells.

5. The method of claim 4, further comprising characterizing the prostate cells
using at least one prostate-specific marker expressed by the prostate cells.

6. The method of claim 5, comprising detecting at least one of a prostate-specific antigen and a prostate-specific membrane antigen.

7. The method of claim 4, further comprising characterizing the prostate cells using a cytokeratin protein marker expressed by the prostate cells.

8. The method of claim 6, wherein at least one of the prostate-specific antigen and the prostate-specific membrane antigen is detected using a nucleic acid probe that specifically binds to the mRNA of said antigen.

9. The method of claim 8, wherein said probe is selected from the group consisting of SEQ. ID. Nos. 1, 2, and 6.

10. The method of claim 8, wherein said probe is selected from the group consisting of SEQ. ID. Nos. 3, 4, 7, and 8.

11. The method of claim 6, wherein at least one of the prostate-specific antigen and the prostate-specific membrane antigen is detected using an antibody that specifically binds to said antigen.

12. The method of claim 3, further comprising characterizing ploidy state of the epithelial cell using at least one centromere specific marker.

13. The method of claim 12, wherein the centromere specific marker comprises a nucleic acid probe that specifically binds to a complementary sequence of the centromere DNA.

14. The method of claim 13, wherein said probe comprises SEQ. ID. No. 5.

15. The method of claim 13, wherein said probe comprises SEQ. ID. No. 10.

16. The method of claim 1, wherein said binding agent

comprises an antibody.

17. The method of claim 16, wherein said binding agent comprises at least two primary antibodies from animals that are capable of binding to different non-rare cell antigens.

18. The method of claim 16, wherein said binding agent comprises a primary antibody from an animal that binds to a non-rare cell, and a secondary anti-antibody from another species than the primary antibody, wherein said secondary anti-antibody binds to the primary antibody.

19. The method of claim 17, wherein the at least two primary antibodies are capable of binding to human non-rare cell antigens, and the binding agent further comprises secondary antibodies capable of binding to the two primary antibodies, wherein the primary antibodies are from a different species than the secondary antibodies.

20. The method of claim 1, wherein subjecting the sample to density gradient separation comprises using at least one density gradient medium having a density of no less than about 1.06 g/ml.

21. A method of detecting cancer cells in a fluid comprising cancer and non-rare cells, which method comprises providing a fluid enriched with cancer cells by the method of claim 1, and analyzing said fluid to detect said cancer cells.

22. A method of detecting prostate cancer cells comprising providing a fluid enriched with prostate cancer cells by the method of claim 4, and analyzing said fluid to detect the prostate cancer cells.

23. The method of claim 3, further comprising determining the number of chromosomes in the cancer cells.

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24. The method of claim 4, further comprising determining the number of chromosomes in the prostate cancer cells.

25. The method of claim 23, including determining the presence of aneuploidy in the cancer cells.

26. The method of claim 3, comprising increasing by at least about 500-fold the concentration of the cancer cells compared to the concentration of the cancer cells to the non-rare cells in the fluid sample.

27. The method of claim 1, wherein the cancer cells are human liver cells, hepatoma cells, or hepatocarcinoma cells.

28. The method of claim 1, wherein said fluid is blood.

29. The method of claim 1 comprising subjecting the second fluid comprising an increased concentration of cancer cells of a second density to a binding agent that binds white blood cells and red blood cells, and removing the bound white blood cells and bound red blood cells from the second fluid to provide a second fluid enriched with the greater density cancer cells.

30. The method of claim 1, wherein the cells are alive during the course of said method.

31. The method of claim 1, wherein the non-rare cells comprise blood cells.

32. The method of claim 31, wherein the blood cells comprise leukocytes and red blood cells.

33. A cancer cell enriched fluid prepared in accordance with the method of claim 1.

34. The method of claim 1, wherein the first density gradient has a density in the range of about 1.068 g/mL to about 1.077 g/mL, and wherein the second density

gradient has a density in the range of about 1.077 g/mL to about 1.085 g/mL.

35. The method of claim 3, wherein the epithelial cancer cells comprise breast
cancer cells.

36. The method of claim 3, wherein the epithelial cancer cells comprise kidney
cancer cells.

37. The method of claim 1, wherein subjecting the second fluid to the binding agent
includes binding the white blood cells and/or red blood cells to magnetic beads.